*INHIBITION OF HUMAN BRAIN PYRUVATE KINASE AND HEXOKINASE BY PHENYLALANINE AND PHENYLPYRUVATE: POSSIBLE RELEVANCE TO PHENYLKETONURIC BRAIN DAMAGE**

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Abstract.—In phenylketonuria high levels of L-phenylalanine are present along with increased levels of phenylpyruvic acid. The present work shows that L-phenylalanine is a competitive inhibitor of human brain pyruvate kinase and phenylpyruvic acid is an inhibitor of human brain hexokinase. The enzymes have approximately the same K_i for these inhibitors in adult and in fetal human brain. However, in the fetal human brain the absolute activities for both enzymes are less than 10 per cent of those found in the adult. Thus, the fetal brain enzymes may be more vulnerable to inhibition by these compounds in the phenylketonurics. The inhibition of human brain pyruvate kinase and hexokinase by L-phenylalanine and phenylpyruvic acid may have a role in the brain damage in phenylketonurics.

In the metabolic disease phenylketonuria there is a marked accumulation of L-phenylalanine (L-Phe) and phenylpyruvic acid (PPA) in the blood. If the disease is not recognized promptly after birth, mental deficiency frequently results. Diets low in L-Phe that are employed at a very early stage after birth may favorably influence a child's mental development. However, this is not so in all cases and the low L-Phe diet can itself be injurious. The enzymatic origin of the phenylalaninemia and high phenylpyruvic acid levels is accounted for, in part at least, by the absence of phenylalanine hydroxylase in the liver of phenylketonuric children. However, there appears to be little information regarding the metabolic attacking point of the damage exerted in the brain at the molecular level by the high L-Phe and PPA levels. This situation emphasizes the need for a deeper understanding of the biochemistry of the disease.

Investigations in my laboratories concerning the control mechanisms of glycolysis demonstrated that one of the hepatic key glycolytic enzymes, pyruvate kinase, was competitively inhibited by an amino acid, L-alanine, and that L-Phe was a weak inhibitor for this liver enzyme.[1] In extending these studies to the brain enzyme, it was observed that L-Phe was a strong inhibitor for rat brain PK activity.[2, 3]

Materials and Methods.—Adult human brain was obtained at operation for removal of brain tumors. Human fetal samples were obtained at therapeutic abortion carried out for psychiatric reasons. For the adult brain the cortical area was studied; in the fetal samples because of the early age group it was only feasible to use whole brain. The human material was obtained at the operating table and kept on ice; it was homogenized approximately 30 min afterwards. Male Wistar rats 200 gm in weight were decapitated,

and the brain cortex was rapidly removed. The preparation of homogenate and supernatant fluid and cell counts was carried out according to methods described previously.[4, 5] The enzyme assay systems were adapted from those used for the liver.[6, 7] Through kinetic studies the optimum levels of the reactants were determined. The assays were carried out in the Gilford 2000 recording spectrophotometer at 37°C and pH 7.4. The final pH and temperature were checked at the termination of each assay. Pyruvate kinase activity was assayed in a reaction mixture containing the following components per ml: glycylglycine buffer, 42 μmoles; $MgSO_4$, 25 μmoles; phosphoenolpyruvate, 1 μmole; ADP, 0.9 μmole; NADH, 0.4 μmole; lactic dehydrogenase, 24 International Units; rat brain supernatant fluid, 0.08 mg tissue equivalent with distilled water added to make a final volume of 3 ml. Hexokinase was assayed in a reaction mixture containing the following components per ml: glycylglycine buffer, 50 μmoles; $MgCl_2$, 7.5 μmoles; glucose, 5 μmoles; ATP, 5 μmoles; NADP, 0.75 μmole; cysteine, 2 μmoles; glucose-6-phosphate dehydrogenase, 0.16 IU; rat brain supernatant fluid, 1.3 mg tissue equivalent with distilled water added to make a final volume of 2.5 ml. As a result of careful adaptation of the kinetic conditions, proportionality with tissue amount added and with time elapsed was obtained. The described assay procedures were followed throughout this work unless otherwise specified. Enzyme activities were calculated as μmoles of substrate metabolized per gm wet weight per hr at 37°C.

Results and Discussion.—Figure 1 shows that the addition of L-Phe to the rat brain pyruvate kinase assay system caused a dose-dependent progressive inhibition with a K_i = 5.8 mM. A Dixon plot shows that this is a competitive inhibition, yielding a K_i = 4.6 mM in this experiment (Fig. 2). The L-Phe inhibition of rat brain pyruvate kinase activity can be readily reversed by increasing the concentration of the substrate, phosphoenolpyruvate.[2] D-Phenylalanine was not inhibitory. The pyruvate kinase inhibitory concentrations are in the same order of magnitude as those reported in the plasma of phenylketonuric patients.[8, 9]

Further studies revealed that adult human brain pyruvate kinase activity was also inhibited by L-Phe, yielding a K_i = 8.5 mM (Fig. 3). Investigation of two

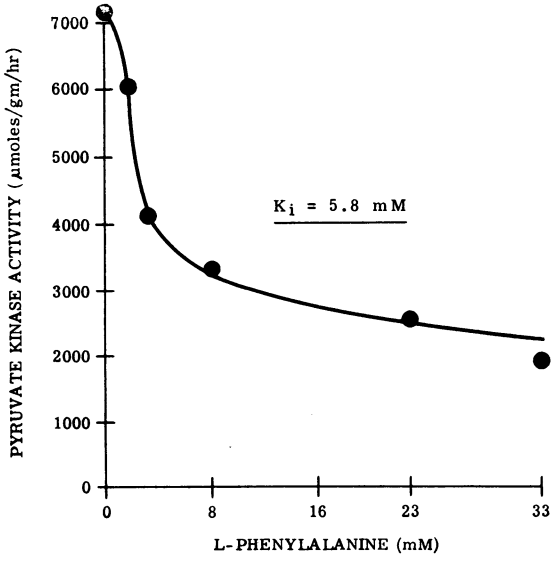

FIG. 1.—Inhibitory effect of L-phenylalanine on pyruvate kinase of adult rat brain cortex: dose-response studies at a PEP level of 0.1 mM.

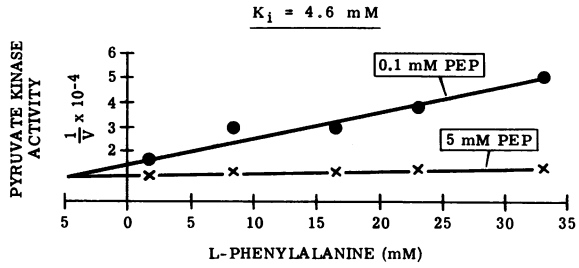

FIG. 2.—Inhibition of rat brain cortex pyruvate kinase activity by L-phenylalanine at two levels of substrate. The Dixon plot indicates competitive inhibition.

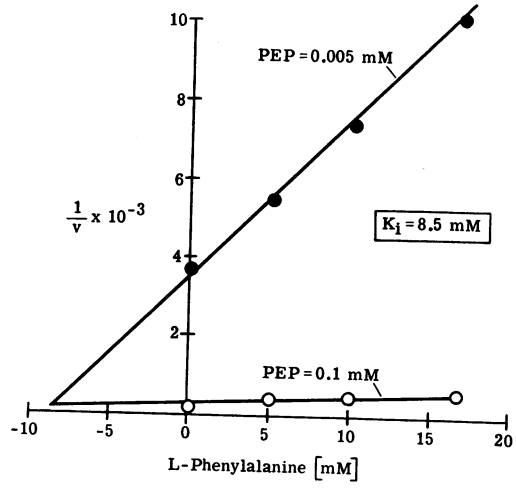

FIG. 3.—Inhibition of pyruvate kinase activity in adult human brain cortex at two levels of substrate.

samples showed that human brain pyruvate kinase activity in a 21-week-old and in a 14.5-week-old fetus was also inhibited by L-Phe with a K_i = 11 mM (Table 1). It is important that the activity of the fetal human brain pyruvate kinase was less than 10 per cent of the activity observed in the adult. Thus, fetal and adult pyruvate kinase exhibit similar kinetic properties (Table 3) and a similar sensitivity to inhibition by L-Phe (Table 1). However, the very low enzyme activity in the fetal brain could be a great deal more vulnerable to inhibition by L-Phe. In the newborn rat the brain PK activity is low, and it rises after delivery to reach the adult level in several weeks (Table 2). If the same applies to the human brain pyruvate kinase after birth, then the infant continues to be more sensitive to L-Phe inhibition than an adult would be.

Phenylpyruvic acid also accumulates in the phenylketonurics, and it was found to be inhibitory to human hexokinase in adult as well as in fetal brain. At all ages the human brain hexokinase showed similar kinetic properties (Table 3) and was about equally sensitive to inhibition by phenylpyruvic acid (Table 1). However, the activity of this enzyme was again much lower in the fetal brain than in the adult, thus making it possibly more vulnerable to inhibition. In further studies it was also shown that L-Phe and PPA are able to inhibit, in a dose-dependent fashion, lactate production from glucose in a cell-free test system and in slices.[10]

TABLE 1. *Effect of L-phenylalanine and phenylpyruvic acid on human brain pyruvate kinase and hexokinase activity.*

		Human Brain Enzymes			
		Pyruvate kinase		Hexokinase	
Age	Cellularity*	Activity† per cell $\times 10^{-7}$	K_i (mM) L-phenylalanine	Activity† per cell $\times 10^{-7}$	K_i (mM) Phenylpyruvic acid
Adult	100	489	8.5	3.80	2.0
Fetal (21 weeks)	520	38	11.0	0.30	6.8
Fetal (14.5 weeks)	684	37	11.0	0.26	4.9

* Number of nuclei counted in millions per gm wet weight of tissue.
† μMoles of substrate metabolized per gm wet weight per hr at 37°C per cell $\times 10^{-7}$.

TABLE 2. *Rat brain pyruvate kinase activity during development.*

Brain	Cellularity*	PK activity per cell†
1 day after birth	294	214
18 days after birth	110	698
Adult animal	120	800

* Number of nuclei counted in millions per gm wet weight of tissue.
† μMoles of substrate metabolized per gm wet weight per hr at 37°C per cell $\times 10^{-7}$.

TABLE 3. *Affinity of human brain enzymes to substrates and cofactors.*

	Pyruvate Kinase K_m (mM)			Hexokinase K_m (mM)		
Human Brain	PEP	ADP	Mg^{++}	Glucose	ATP	Mg^{++}
Adult	0.07	0.29	0.91	0.75	1.06	2.20
Fetal (21 weeks)	0.06	0.46	0.83	0.80	1.11	1.80

In view of the inhibition of two key glycolytic enzymes and the overall glycolysis by metabolites that accumulate in phenylketonuric patients, it is conceivable that the metabolic damage can be caused by these compounds through several attacking points. Thus, a decrease in brain glycolysis should result in a decrease in energy production; it should curtail the biosynthesis of lipids and complex lipids which are required for the biosynthesis of structural elements of the developing brain. In fact, a decrease in cerebroside biosynthesis has been demonstrated in L-Phe-loaded primates.[11] Furthermore, a decrease in brain pyruvate kinase activity could lead to a decline in the production not only of ATP but also of other nucleoside triphosphates. It was described elsewhere that in addition to ATP other nucleotides, such as GTP, UTP, ITP, CTP, and TTP, can be produced in the pyruvate kinase reaction in liver,[2] and this also applies to the brain enzyme as shown in Table 4. As a result, an inhibition of brain pyruvate kinase activity by L-Phe may cause a decrease in the rate of production and levels of ATP, GTP, UTP, and other nucleoside triphosphates which are vital in lipid, protein, and DNA and RNA synthesis. Since brain development entails a gradual increase in the levels of ATP, GTP, and UTP,[12] an inhibition of brain pyruvate kinase might lead to a serious interference with biosynthetic cellular functions as well as cellular multiplication.

A further point of possible therapeutic interest is the fact that L-Phe is a competitive inhibitor and in the *in vitro* assay system the inhibition of brain pyruvate

TABLE 4. *Affinity of rat brain pyruvate kinase* to nucleoside diphosphates.*

Nucleotides	K_m (mM)	Pyruvate kinase activity	Per cent of activity with ADP
ADP	0.26	8 200	100
GDP	1.00	7 300	89
IDP	1.40	4 700	57
UDP	0.91	2 600	32
CDP	1.28	1 400	17
TDP	0.22	300	4

* μMoles of substrate metabolized per hr per gm wet weight of tissue at 37°C.

kinase by L-Phe[2, 3] or the glycolysis by L-Phe or PPA[10] is readily reversed by raising the phosphoenolpyruvate level. It is possible that the high carbohydrate diet might be able to protect the brain from the potential damage of high phenylalanine levels, thus avoiding the use of low phenylalanine diets with their potential problems.

Abbreviations: L-Phe, L-phenylalanine; PPA, phenylpyruvic acid; PK, pyruvate kinase; PEP, phosphoenolpyruvate.

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